Enhanced Integumental and Ocular Amelanosis Following the Termination of Cyclosporine Administration*

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The Smyth delayed amelanotic line of chickens display symptoms commonly associated with human vitiligo. Administration of the immunosuppressive compound, cyclosporine, significantly delayed the mean age of onset and incidence of integumental pigment losses in this mutant line of vitiliginous chickens. Associated ocular pathology was also less severe in treated chicks. Termination of cyclosporine administration resulted in enhanced integumental and choroidal amelanosis, choroidal inflammation, and chorioretinal damage beyond that observed in nontreated controls. These results suggest that withdrawal of cyclosporine in treatment of this spontaneous autoimmune disease may exacerbate associated symptoms. *J Invest Dermatol 88:758–761, 1987*

The Smyth line of chickens expresses a syndrome analogous to human vitiligo, characterized by a progressive loss of melanin pigment [1,2]. Commonly shared features include a spontaneous postnatal loss of integumental and ocular melanin via melanocyte destruction, hypothyroidism, feather or hair defects resembling alopecia areata, visual impairment, and an increased incidence of autoantibodies [1,3–11]. At hatching, Smyth line chicks are phenotypically normal and possess heavily melanized plumage. As early as 5 weeks of age, Smyth line chicks may exhibit a spontaneous loss of melanin pigment in all structures of epidermal and possibly dermal origin [1]. In addition, melanin is lost in the choroid and retinal pigment epithelium (RPE) of the eye. Frequently associated with ocular amelanosis is a high incidence of neural retinal degeneration, mononuclear leukocyte invasion, and blindness [3–5]. Irregularities in melanosomal membranes represent the proposed basic genetic defect in the Smyth line. It is this defect that is hypothesized to initiate the destruction of melanocytes [12].

The vitiliginous condition in the Smyth line and humans has been proposed as an autoimmune disease [1,13–15]. Neonatal bursectomy or corticosterone administration significantly reduces the frequency and severity of integumental and ocular pigment losses [16,17], suggesting that the vitiligo of the Smyth line could be manipulated by immunosuppression. Cyclosporine (CYS), a potent inhibitor of interleukin-1 and -2 release, has a profound influence on helper/inducer T-lymphocyte function [18–21]. This compound has been effective in preventing allograft rejection, graft-vs-host disease and in treating some experimentally induced and spontaneously occurring autoimmune diseases [22,23]. Because an immune component had been hypothesized in the pathogenesis of the Smyth line vitiligo, we administered CYS to evaluate its potential to suppress the development of the line-associated amelanosis.

MATERIALS AND METHODS

Smyth delayed amelanotic line chicks were obtained from parental colonies developed and maintained at the University of Massachusetts. All chicks were reared in electrically heated batteries with raised wire flooring. Feed and water were available ad libitum. A 16 h light/8 h dark photoperiod was provided by fluorescent lighting for the duration of the study. At hatching, chicks were randomly assigned to one of 4 treatment groups. Groups CYS-4 (n = 50), CYS-8 (n = 50), and CYS-12 (n = 30) received CYS injections (intramuscular, 40 mg CYS/kg body weight, olive oil vehicle, 3×/week) [24] for 4, 8, and 12 consecutive weeks, respectively. Control chicks, CYS-0 (n = 50), received olive oil injections only, as did all treated groups following the termination of CYS administration. Integumental amelanosis was monitored weekly to determine the onset and frequency of pigment loss. Chicks were classified as amelanotic when emerging feathers were devoid of pigment. Chorioretinal amelanosis and inflammation were determined at 8 and 12 weeks of age. Randomly selected chicks were enucleated under deep anesthesia (2% sodium pentobarbital) and ocular tissues prepared for histologic evaluation according to previously reported procedures [25]. Following removal of the anterior segment, the eyecup was immersed in fixative (1.5% paraformaldehyde, 1.25% glutaraldehyde in 0.12 M phosphate buffer (pH 7.4) with 0.2 mM $CaCl_2$). Following 2.5 h

Manuscript received July 7, 1986; accepted for publication November 3, 1986.

This research was funded in part by National Institutes of Health grant AM25252 and the Massachusetts Experiment Station.

*A part of these data were reported in a preliminary abstract (Fite KV, Pardue SL, Bengston L, Smyth JR Jr: Withdrawal of cyclosporine A enhances avian autoimmune uveitis. Invest Ophthalmol Vis Sci 26:99, 1985).

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Abbreviations:
CYS: cyclosporine
OS: Obese strain
RPE: retinal pigment epithelium

0022-202X/87/$03.50

of fixation, 1 mm-diameter tissue punches were taken from the peripapillary region. Tissue samples remained in fixative overnight (4°C), were rinsed in 0.12 M phosphate buffer with 0.02 mM $CaCl_2$ and 8% dextrose the following day, and then placed in 2% OsO_4 with 0.12 M phosphate buffer and 7.5% dextrose for 1 h. Following straining, tissues were dehydrated and embedded in Polybed 812. One micron-thick semithin sections were taken from all tissue punches and rated for choroidal amelanosis, choroidal inflammation, and RPE histopathology. Sections were analyzed without knowledge of treatment by 2 experienced neurohistologists according to the following indices: (1) choroidal amelanosis (scale 0–4, where 0 = no pigment loss, 4 = complete depigmentation), (2) choroidal inflammation (scale 0–5, where 0 = no inflammation, 5 = massive inflammatory response), and (3) RPE and retinal pathology (scale 0–6, where 0 = normal, 6 = complete destruction of RPE and photoreceptors). Following enucleation, chicks received a lethal injection of 2% sodium pentobarbital (i.v.). Cumulative integumental amelanosis percentages represent data obtained from all the chicks, including those that were selected for histologic evaluation. Therefore, those sacrificed at 8 or 12 weeks of age remained classified as amelanotic or normally pigmented in all subsequent analyses. Consequently, the data reflect a bias toward a lower frequency of amelanosis.

RESULTS

Mean onset of integumental amelanosis was delayed by the administration of CYS in CYS-8 ($p \leq .05$) and CYS-12 ($p \leq .01$) chicks (Fig 1). The extent of the delay in onset was positively correlated with the length of CYS administration ($r = 0.986$). CYS-0 chicks exhibited a mean onset age of amelanosis that preceded that of CYS-12 chicks by approximately 5 weeks. Initial pigment losses were detected at 5, 7, 8, and 9 weeks of age for groups CYS-0, CYS-4, CYS-8, and CYS-12, respectively (Fig 2). The CYS-0 chicks exhibited a greater incidence ($p \leq 0.05$) of amelanosis than other treatment groups at 6 weeks of age (Fig 2). These differences persisted through weeks 7, 8, and 12 when compared with CYS-4 ($p \leq 0.05$), CYS-8 ($p \leq 0.05$), and CYS-12 ($p \leq 0.01$) chicks, respectively. At 8 weeks of age, CYS-4 chicks displayed a greater ($p \leq 0.01$) incidence of amelanosis when compared with all other groups. The CYS-4 chicks continued to exhibit greater ($p \leq 0.01$) amelanosis than CYS-0 and CYS-12 chicks for the duration of the experiment. Differences ($p \leq 0.05$) from CYS-8 chicks were maintained through week 13. From 11–16 weeks of age, CYS-8 displayed a greater incidence ($p \leq 0.05$) of amelanosis than CYS-12 chicks.

Ocular pathogenesis was delayed during CYS administration; however, cessation of CYS resulted in greater tissue damage. Choroidal pigment loss followed the trend observed for integumental amelanosis (Fig 3). At 8 weeks, significantly greater (p

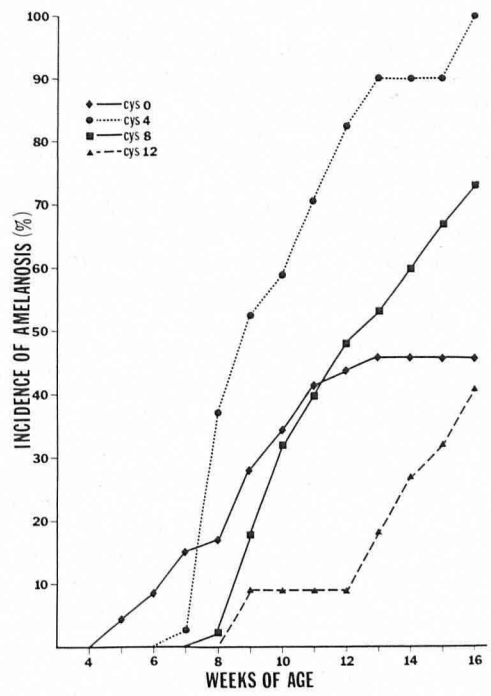

Figure 2. Cumulative percent incidence of integumental amelanosis as influenced by CYS.

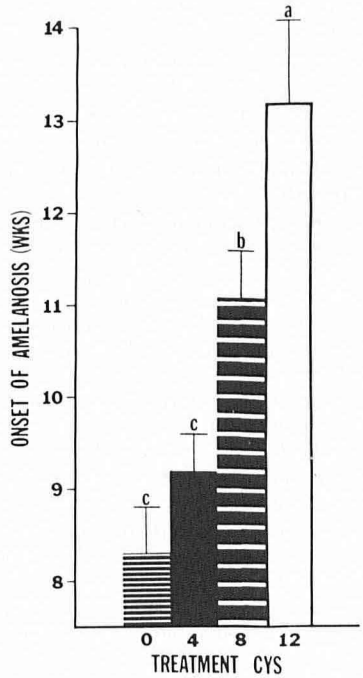

Figure 1. Influence of CYS on the onset of integumental amelanosis. Mean age of initial integumental amelanosis in SDA chicks following CYS administration for 0, 4, 8, and 12 weeks. Values represent the mean ± SEM. Means possessing different letters differ significantly at $p < 0.05$.

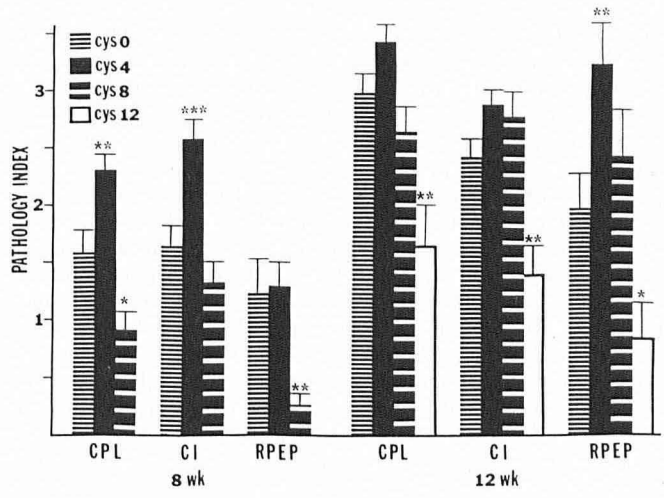

Figure 3. Ocular pathology in CYS-treated SDA chicks. Abbreviations: *CPL,* choroidal pigment loss; *CI,* choroidal inflammation; RPEP, retinal pigment epithelium pathology. Values represent the mean ± SEM (n = 12–15, 4 sections evaluated per individual; n = 6 for CSY-12-treated chicks). * = $p < 0.05$, ** = $p < 0.01$, and *** = $p < 0.001$ when compared with controls (CYS-0).

≤ 0.01) melanin losses occurred in the choroids of CYS-4 chicks when compared with controls. However, CYS-8 chicks exhibited less ($p \leq 0.05$) choroidal amelanosis when compared with controls (an apparent therapeutic effect of CYS). All groups demonstrated marked increases in choroidal pigment losses at 12 weeks of age when compared with 8-week values. At this time, choroidal pigment losses were reduced ($p \leq 0.01$) in CYS-12 chicks when compared with controls. Choroidal inflammation followed a similar pattern to that observed in choroidal amelanosis. CYS-4 chicks exhibited enhanced ($p \leq 0.001$) inflammation at 8 weeks of age. At 12 weeks, choroidal inflammation associated with CYS-12 chicks was reduced ($p \leq 0.01$). Finally, RPE pathology indices were lower in CYS-8 chicks ($p \leq 0.01$) at 8 weeks and in CYS-12 chicks ($p \leq 0.05$) at 12 weeks. A more severe ($p \leq 0.01$) pathologic condition existed in the RPE of CYS-4 chicks at 12 weeks.

DISCUSSION

Pathology associated with the Smyth line was ameliorated by the administration of CYS. In addition, pathologic changes in ocular tissue were also limited when compared with untreated controls. These findings are in agreement with reports that CYS prevented the development of experimentally induced autoimmune uveitis [26] and collagen arthritis in rats [27]. In the present study, the reduction in Smyth line-associated pathology (therapeutic effect) was dependent on the length of time over which CYS treatment occurred. Chicks receiving CYS from hatching to 4 weeks of age exhibited a slight increase in the mean age of onset and a 2-week delay in the initiation of integumental amelanosis. The earliest age at which integumental amelanosis occurred in controls was 5 weeks. Because CYS presumably inhibits the proliferation of antigen-primed T lymphocytes, administration of CYS from 0–4 weeks of age may have coincided with a period of limited antigenic activation of T lymphocytes by melanocytic antigen(s). Therefore, prolonged delays in the development of amelanosis would not be expected as a result of this treatment schedule. A similar hypothesis was proposed by Like and coworkers to explain the age-dependent effects of CYS on the development of an autoimmune diabetes mellitus in BB/W rats [28].

Although we anticipated that treatment with CYS from 0–4 weeks of age would be the least effective in ameliorating the development of amelanosis, the apparent exacerbation of SDA-line pathology in CYS-4 chicks following the cessation of CYS was unexpected. Integumental and choroidal amelanosis, choroidal inflammation, and RPE abnormalities were significantly enhanced following treatment with CYS for 4 weeks. The incidence of integumental amelanosis increased from 2.7% to 37.1% in CYS-4 chicks from 7–8 weeks of age, whereas in controls, increases from 14.9% to 17.0% were noted during the same time period. Similar enhanced rates of integumental pigment loss occurred following the cessation of CYS administration in CYS-8 and CYS-12 chicks. In CYS-4 chicks, a marked increase in integumental amelanosis did not occur until 4 weeks subsequent to the last CYS injection. However, in CYS-8 and CYS-12 chicks, dramatic increases in pigment losses were evident as early as 1 week following the end of CYS treatment. These differences would appear to be directly related to the mean age of onset of amelanosis observed in the control population (8.3 weeks). Previous research has shown that as the Smyth line chick ages, melanosomal defects become more severe and may reflect a potential increase in antigenicity [13]. Because CYS-mediated immunosuppression is reversible, an increase in antigenicity with increasing age could explain the reduction in latency of onset and enhancement above control levels.

It is interesting to note that in the Obese strain (OS) chicken, a spontaneous autoimmune thyroiditis model that resembles Hashimoto's disease in humans, CYS administration also enhances the development of an autoimmune disease [29]. Embryonic administration of CYS to OS embryos resulted in a significant increase in lymphoid infiltration of the thyroid and a higher incidence of thyroglobulin autoantibody. In addition, enhancement of experimentally induced arthritis in rats occurred when CYS treatment was initiated during the preclinical phase or when the disease was established [27]. In the present study, exacerbation of symptoms occurred following the termination of CYS treatment, whereas in the rat arthritis model, enhanced pathology occurred during CYS administration. It is apparent that CYS may enhance symptoms associated with spontaneous autoimmune diseases such as Smyth line vitiligo and with experimentally induced autoimmune disorders. Additional research will be required to determine the precise role of the temporal component in the enhancement of symptoms associated with the administration of this drug in vitiligo of the Smyth chicken.

We thank Dr. David Winter and Sandoz Ltd. for the gift of the cyclosporine and acknowledge the technical assistance of S. Finerman-Bierly, D. Maslak, D. Hayden, and H. Khouri.

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